frontiers | Frontiers in Animal Science

TYPE Original Research
PUBLISHED 20 November 2025
DOI 10.3389/fanim.2025.1710910

OPEN ACCESS

EDITED BY
Ricardo Ekmay,
Arbiom, United States

REVIEWED BY
Jianping Quan,
South China Agricultural University, China
Emilia König,
University of Helsinki, Finland

*CORRESPONDENCE
Barbara U. Metzler-Zebeli
barbara.metzler@vetmeduni.ac.at

RECEIVED 22 September 2025
REVISED 24 October 2025
ACCEPTED 04 November 2025
PUBLISHED 20 November 2025

CITATION
Hartinger K, Lerch F, Yosi F, Vötterl JC and Metzler-Zebeli BU (2025) Pig feed as a source of bacterial DNA and its potential impact on porcine gut microbiota studies.
Front. Anim. Sci. 6:1710910.
doi: 10.3389/fanim.2025.1710910

Pig feed as a source of bacterial DNA and its potential impact on porcine gut microbiota studies

Kristina Hartinger[1,2], Frederike Lerch[1,2], Fitra Yosi[1,2,3], Julia C. Vötterl[1,2] and Barbara U. Metzler-Zebeli[1,2]*

[1]Centre for Systems Transformation and Sustainability of Veterinary Medicine, Clinical Department for Farm Animals and Food Safety Systems, University of Veterinary Medicine, Vienna, Austria, [2]Christian-Doppler Laboratory for Innovative Gut Health Concepts of Livestock, Centre for Animal Nutrition and Welfare, Clinical Department for Farm Animals and Food Safety Systems, University of Veterinary Medicine Vienna, Vienna, Austria, [3]Department of Animal Science, Faculty of Agriculture, University of Sriwijaya, Palembang, Indonesia

Aside from being a source of energy and nutrients, a pig's diet is also a source of microbial DNA, which may be a confounding factor in porcine gut microbiota studies based on DNA sequencing. Therefore, this small-scale pilot study aimed to investigate gene copy numbers, diversity, and taxonomic composition of bacterial DNA present in complete feeds for sows and piglets, including gestation, lactation, and prestarter diets, as well as a milk replacer, and to compare the bacterial communities in feeds for piglets with those in the gastric digesta of piglets consuming these feeds. Total DNA was extracted from feed and gastric samples of piglets for 16S rRNA gene amplicon (V3–V4 region) sequencing. The results showed that the feeds carried a high amount of bacterial DNA, ranging from 8.3 to 9.0 $\log_{10}$ gene copies/g feed. Beta-diversity analysis further indicated clear separation between the bacterial communities in the cereal-based gestation, lactation, and prestarter diets and the milk replacer, whereas alpha-diversity was similar among feeds. Taxonomic evaluation demonstrated that cereal-based feeds were dominated by *Pantoea*, *Pseudomonas*, *Paenibacillus*, and *Erwinia*, making up 41%–68% of all reads, whereas the milk replacer was dominated by *Streptococcus* and *Lactococcus*, with 57.4% and 13.2% relative abundance, respectively. Comparison of the bacterial communities in feed to those in the gastric digesta of piglets on days 3, 7, 14, 21, 28, 31, and 35 of life showed that, although mostly low in abundance, many taxa found in the feed were detectable in gastric digesta. A comparison at the species level is more appropriate to estimate the proportional contribution of bacteria in feed to the total bacterial DNA in gastric digesta. Overall, these data provide valuable insight into the bacterial DNA load and diversity in pig feeds and demonstrate that bacterial DNA found in gastric digesta may be influenced to a small degree by bacteria from the feed.

KEYWORDS

bacterial DNA, milk replacer, cereal-based diets, pig feed, microbiota

1 Introduction

There is great interest in investigating the effect of diets and specific dietary ingredients on the porcine gut microbiota at various stages of life (Bonde et al., 2025; Stanley et al., 2025). Several factors, such as environment, genetics, and exact dietary composition, have been suggested to explain the observed variation in dietary effects on the porcine gut microbiota or microbiota associated with a specific host phenotype (i.e., high feed efficiency) among studies (Gardiner et al., 2020; Tardiolo et al., 2025). Many studies on the gut microbiota are based on 16S rRNA amplicon or shotgun metagenome sequencing of total DNA extracted from gastrointestinal digesta or feces (Yosi et al., 2024; Tardiolo et al., 2025). In doing so, DNA is extracted from all cells found in digesta or feces, including DNA from microbes, plants (feed residuals), and the exfoliated enterocytes of the host animal. Pig feeds are comprehensively characterized regarding their nutrients to avoid nutrient oversupply, associated environmental pollution, and unnecessary costs while meeting nutritional requirements as precisely as possible (Cheli et al., 2012). However, it is often overlooked that feed comes with its own microbiota and thus microbial DNA, which is extracted as part of the total DNA (Karlsen et al., 2022). These microbes may have a wide variety of origins from before or after feed preparation (e.g., storage), and the feed matrix may serve as a vehicle for their transmission to the gut (Maciorowski et al., 2007; Jackman et al., 2020). Although microbes may be killed during processing, as intended by pelleting or expansion of pig feed, their DNA or DNA fragments remain, as previously shown for fish feed (Karlsen et al., 2022). Thermal processing of feed targets the viability of microbes to inhibit pathogens present in the feed from causing disease. Nevertheless, the DNA of dead bacteria is still present in feed and may be detected in digesta, especially of the upper gut after ingestion by the animal. Therefore, this small-scale pilot study aimed to examine the bacterial load, diversity, and taxonomic composition of the DNA in commercial complete feeds for sows and piglets, including three cereal-based diets and one milk replacer, and to compare the bacterial communities in the feeds for piglets with those in the gastric digesta of piglets consuming these feeds. We hypothesized that considerable amounts of bacterial DNA were present in the four types of pig feeds, with taxonomy and diversity varying depending on the type of feed. We further hypothesized that the major taxa found in the feed would be detectable in the stomach content of piglets. The data for the gastric microbiota were previously published at the genus level (Yosi et al., 2024) and were reanalyzed at the species level for this study to allow comparison between the relative abundances of amplicon sequence variants (ASV) found in the piglet feeds and gastric digesta.

2 Materials and methods

2.1 Sample collection

All procedures requiring animal handling and treatment were approved by the institutional ethics committee of the University of Veterinary Medicine Vienna and the national authority in accordance with the Law for Animal Experiments in Austria (GZ 2020-0.437.208).

The feeds investigated in this small-scale study comprised two commercial sow feeds (gestation and lactation diets) and two piglet feeds (milk replacer and prestarter diet). Feed samples (n = 4 per feed type) were collected at the pig facility of the University of Veterinary Medicine Vienna (VetFarm, Austria) in replicate batches 1 and 2 (n = 2 per feed and replicate batch) of an experiment with sows from late gestation throughout lactation, as well as their litters (Yosi et al., 2024). The replicate batches started two months apart. For each sample type and time point, several subsamples from feed bags were taken and mixed separately for each type of feed before being stored at −20 °C. The ingredient and chemical composition of the cereal-based feeds are shown in Tables 1 and 2.

Detailed information on the management of sows and piglets and the collection of gastric digesta samples can be found in Yosi et al. (2024). Briefly, gastric digesta samples of piglets were collected on days 3, 7, 14, 21, 28, 31, and 35 of life over the course of two replicate batches. Ten piglets (five males and five females) were sampled on each day of life in each of the two replicate batches, resulting in 20 observations for each day of life.

Piglets could suckle freely during the suckling period of 28 days. The milk replacer (NuriStart Sweet, BIOMIN Holding GmbH, part of dsm-firmenich, Getzersdorf, Austria; Table 2) was offered in liquid form from day 3 to 23 of life by mixing the powder with warm water (45°C) at a ratio of 500 g/L (w/v) by hand. The stainless-steel feeders were cleaned before they were refilled with the liquid milk replacer. Each litter received a minimum of 1,000

TABLE 1 Ingredient composition (%) of gestation, lactation, and pre-starter diet (adapted from Yosi et al., 2024).

Ingredients	Gestation diet[1]	Lactation diet[2]	Prestarter[3]
Barley	50.0	20.0	29.7
Wheat	8.1	10.0	10.0
Wheat, hydrolyzed	–	–	9.9
Corn	10.0	29.0	10.8
Wheat bran	15.8	6.4	–
Soybean meal	4.2	9.3	–
Full fat soybeans	–	–	14.1
Sweet whey powder	–	–	3.0
Potato protein	–	–	5.0
Rapeseed meal	–	5.0	–
Dried pulp	4.0	1.5	–
Commercial breeding premix	2.8	3.5	–
Lignocellulose	–	0.5	0.9
Palm kernel	–	–	2.0

(Continued)

TABLE 1 Continued

Ingredients	Gestation diet[1]	Lactation diet[2]	Prestarter[3]
Dried vinasse[4]	1.5	–	1.9
Dextrose	–	–	5.0
Lactose	–	–	3.0
Apple pomace	1.5	1.0	–
Bakery products	1.2	12.8	–
Hay cobs	0.5	–	–
Oil	0.3	0.5	–
Rapeseed oil	–	–	0.5
Lysine HCL	–	0.2	0.7
Threonine	–	0.06	0.3
Methionine	–	0.02	0.3
Tryptophane	–	–	0.1
Limestone (calcium carbonate)	–	0.2	0.6
Sodium chloride	0.03	–	0.5
Mono calcium phosphate	–	–	1.4
Magnesium phosphate	–	–	0.2
Vitamin E	0.02	0.02	–
Vitamin/trace element premix	–	–	0.4

[1]Vitamin and mineral composition per kg feed: 9.600 IE of Vitamin A, 1.600 IE of Vitamin D3, 156 mg of Vitamin E, 82 mg of Fe as Iron-(II)-sulfate, monohydrate, 12 mg of Cu as Copper-(II)-sulfate pentahydrate, 90 mg of Zn as zinc oxide, 2.6 mg of Mn as manganese -(II)-oxide. Technological additives: 700 FTU 6-phytase, 60 mg of butylated hydroxytoluene.
[2]Vitamin and mineral composition per kg feed: 12.000 IE of Vitamin A, 2.000 IE of Vitamin D3, 170 mg of Vitamin E, 100 mg of Fe as iron-(II)-sulfate monohydrate, 15.1 mg of Cu as Copper-(II)-sulfate pentahydrate, 110 mg of Zn as zinc oxide, 0.7 mg of Mn as manganese-(II)-oxide. Technological additives: 880 FTU 6-phytase, 70 mg of butylated hydroxytoluene.
[3]Vitamin and mineral composition per kg feed: 16.000 IE of Vitamin A, 2.000 IE of Vitamin D3, 150 mg of Vitamin E, 4.0 mg of Vitamin K3, 2.8 mg of Vitamin B1, 8.2 mg of Vitamin B2, 5.0 mg of Vitamin B6, 50 mg of Vitamin B12, 60 mg of Nicotinic acid, 20 mg of Pantothenic acid, 500 mg of choline chloride, 1050 mcg of Folic acid, 150 mcg of Biotin, 124 mg of Fe as iron-(II)-sulfate monohydrate, 80 mg of Mn as manganese-(II)-oxide, 3.1 mg of I as calcium iodate, 121 mg of Zn as zinc oxide, 0.45 mg of Se as sodium selenite, 124 mg of Cu as Copper-(II)-sulfate pentahydrate. Technological additives: 250 FTU phytase (4a16), 100 mg of butylated hydroxytoluene.
[4]CITROFEED, dried residues from citric acid production.

mL per day (500 mL at 08:00 and 500 mL at 15:00 h), and more when piglets finished their portion. The milk replacer was gradually mixed with the prestarter diet from days 24 to 26 of life, starting with a ratio of 70:30 (w/w) on day 24, 50:50 (w/w) on day 25, and 30:70 (w/w) on day 26, respectively, and provided in mash form. After that, the litters were offered 100% of the prestarter diet as mash on day 27 and until weaning on day 28. It was fed in dry form after weaning from day 28 to 35 of life. The cereal-based prestarter diet was formulated to provide adequate nutrients from the week before weaning to one week after weaning (NRC, 2012). Intake of milk replacer was estimated on a pen basis during the suckling period (Yosi et al., 2024). The proximate nutrient composition of the feeds was analyzed by a commercial feed laboratory (Feed Laboratory Rosenau, Haag, Austria) according to standard procedures (Naumann and Bassler, 2012).

On each sampling day, piglets were deeply sedated with azaperone (Stresnil 40 mg/mL, 0.025 mL/kg body weight, Elanco Tiergesundheit AG, Bad Homburg, Germany) and ketamine (Narketan 100 mg/mL, 0.1 mL/kg body weight, Vetoquinol Österreich GmbH, Vienna, Austria), and subsequently euthanized via intracardiac injection with embutramide (T61, 0.1 mL/kg body weight, Intervet GesmbH, Vienna, Austria). Piglets were exsanguinated by cutting the neck. The abdomen was then opened, and the entire gut was removed aseptically. The stomach was identified, clamped, and separated. The entire gastric content was transferred to a sterile container and homogenized with a sterile spatula. Aliquots of the homogenized digesta were snap-frozen in liquid nitrogen and stored at −80°C.

2.2 DNA extraction, sequencing, and bioinformatics

Total DNA was extracted from feed samples in the same way as from gastric digesta (Yosi et al., 2024) using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany). Modifications to the original extraction protocol included an additional heating step for 10 min at 65°C and homogenization of the samples using the SpeedMill Plus System (Analytik Jena GmbH, Jena, Germany). The DNA concentration in each eluate was quantified using the Qubit DNA HS Assay Kit on the Qubit 4 Fluorometer (Thermo Fisher Scientific Inc., Waltham, United States).

Absolute quantification of total bacteria in feeds was performed on a qTOWER real-time PCR system (Analytik Jena GmbH, Jena, Germany) using a previously reported primer set (Muyzer et al., 1993) and amplification conditions (Metzler-Zebeli et al., 2023). The total reaction volume of 10 µL consisted of 0.5 ng DNA, 2.5 µL innuMIX qPCR DSGreen Standard (Analytik Jena GmbH), 300 nM each of forward and reverse primers, and DEPC-treated water (G-Biosciences, St. Louis, MO, USA) in a 96-well plate. Following an initial denaturation at 95°C for 2 min, 40 cycles of 95°C for 30 s and primer annealing and elongation at 60°C for 60 s were performed. Subsequently, melting curve analysis was performed with an increment of 0.1°C/s from 55°C to 95 C, with fluorescence measurement at 0.1°C intervals.

Standard curves were prepared by making serial dilutions (10^{10} to 10^{3} molecules/µl) of purified and quantified PCR products generated using pooled DNA from the pig feedstuffs. Standards were run on the same plate in triplicate, while samples and negative controls without template DNA were included in duplicate. The amplification efficiency, which was calculated according to the following equation: $E = 10^{-1/slope}$, and the coefficient of determination were 109% and 0.994, respectively. The final gene copy numbers of total bacteria per gram of feed sample were calculated using the following equation:

$$(QM \times C \times DV)/(S \times V),$$

TABLE 2 Chemical composition of diets for sows and piglets (adapted from Yosi et al., 2024).

Chemical composition	Gestation diet	Lactation diet	Pre-starter	Milk replacer[1]
Dry matter (DM), %	90.4	89.3	91.9	91.6
Ash, % DM	5.7	6.6	5.1	6.8
Crude protein, % DM	14.7	17.8	18.2	23.3
Crude fiber, % DM	6.5	4.6	4.9	1.9
Ether extract, % DM	5.0	4.9	7.3	10.3
Starch, % DM	44.3	46.7	42.5	25.4
Metabolizable energy, MJ/kg DM	14.2	14.9	15.4	16.9

[1]NURIstart SWEET, BIOMIN GmbH, Getzersdorf, Austria. Ingredient composition: wheat flour, whey protein concentrate, whey powder, extruded soybeans, puffed corn, rolled oats, soy protein concentrate, sugar, dextrose, palm oil, monocalcium phosphate, coconut oil, sodium chloride, calcium carbonate, salmon oil. Vitamin and mineral composition per kg feed: 16.000 IE of Vitamin A, 2.000 IE of Vitamin D3, 150 mg of Vitamin E, 200 mg of Vitamin C, 195 mg of Fe as iron-(II)-sulfate monohydrate, 2 mg of I as calcium iodate, 0.45 mg of Se as sodium selenite, 60 mg of Mn as manganese-(II)-oxide, 140 mg of Cu as Copper-(II)-sulfate pentahydrate, 100 mg of Zn as zinc oxide. Technological additives: 200 FXU of endo-1,4-beta-xylanase, 1.000 FYT of 6-phytase, 400 mg of sepiolite, 3.000 mg of citric acid, 0.07 mg of propyl gallate, 1 mg of butylated hydroxytoluene.

where QM is the quantitative mean of the copy number, C is the DNA concentration of each sample, DV is the dilution volume of extracted DNA, S is the DNA amount (ng) subjected to analysis, and V is the weight of the sample (g) subjected to DNA extraction.

Together with the DNA extracted from the gastric digesta samples (Yosi et al., 2024), the DNA extracts from feeds were sent to an external laboratory (Novogene, Cambridge, UK) for targeted 16S rRNA amplicon sequencing (V3–V4 hypervariable region), which included library preparation (NEBNext Ultra II DNA Library Prep Kit, Illumina, San Diego, CA, USA). The 16S rRNA gene was amplified using primers 341F-ill (5′-CCTACGGGNGGCWGCAG-3′) and 806R-ill (5′-GACTACHVGGGTATCTAATCC-3′). Equimolar pools of samples were sequenced to generate 250 bp paired-end raw reads on the NovaSeq 6000 platform (Illumina). Demultiplexing and trimming of the raw sequences were performed by Novogene.

The bioinformatic analysis was conducted in accordance with the protocol described in Yosi et al. (2024). Raw sequencing reads (Fastq files) were independently processed, aligned, and categorized using the Divisive Amplicon Denoising Algorithm 2 (version 1.26.0) (Callahan et al., 2016), implemented in RStudio (version 1.4.1106). First, the quality profiles of the forward and reverse reads were evaluated. Subsequently, the total length of the reads was cut to 220 nucleotides with a maximum error rate of 5 for both the forward and reverse reads, respectively, to account for degradation of the quality score for the remaining nucleotides using the filterAndTrim function (truncQ = 5). Ambiguous reads and reads exceeding the probabilistic estimated error of two nucleotides were removed from the amplicon set. Following dereplication of the filtered data and estimation of the error rates, ASV were inferred (Callahan et al., 2016). The inferred forward and reverse sequences were merged, paired sequences not matching perfectly (to control against residual errors) were removed, and the sequence table was generated. The sequence reads were then filtered, and chimeras were eliminated using the removeBimeraDenovo command. Subsequently, taxonomy was assigned using the SILVA 138.2 rRNA database for bacteria (Quast et al., 2013) with a 3% dissimilarity threshold.

Alpha diversity (Shannon, Simpson) and species richness (observed features) were determined using phyloseq (version 1.42.0). At the genus and ASV levels, the raw read counts from the various taxa were summed and compositionally normalized so that each sample summed to 1. The ASV within the five most abundant bacterial genera in the four feeds, including *Pantoea*, *Paenibacillus*, *Pseudomonas*, *Streptococcus*, and *Lactococcus*, were extracted from the ASV table for the feed and gastric digesta and statistically analyzed.

2.3 Statistical analysis

Normal distribution of the residuals for the generated data was tested using the Shapiro–Wilk test with the UNIVARIATE procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). If residuals were not normally distributed, data were transformed using the Box–Cox method and the Transreg procedure in SAS.

Afterwards, gene copy numbers of total bacteria, alpha-diversity indices, and relative bacterial abundances in feed samples were subjected to ANOVA using the PROC MIXED procedure of SAS, with type of feed as a fixed effect and time point of sample collection as a random effect. For the comparison of bacterial genera and ASV in the gastric digesta of piglets, a random model was used to investigate the fixed effect of increasing age of piglets. The random effect was animal. Day of life nested within replicate batch and litter represented the experimental unit.

For both random models, the probability of difference option in SAS was used to perform pairwise comparisons among least squares means. The raw P values for the relative abundances of bacterial genera and ASV were adjusted using the Bonferroni correction (Lin and Peddada, 2020). Data are expressed as least squares means ± standard error of the mean. Significance was defined at $P \leq 0.05$ and trends at $P \leq 0.10$.

Permutational multivariate analysis of variance (PERMANOVA, Bray–Curtis distance) was used to assess dissimilarities between the bacterial community structures in feeds and gastric digesta of piglets

using the 'adonis function' in vegan (Oksanen et al., 2022). The two-dimensional non-metric multidimensional scaling (NMDS) ordination plots were created with the metaMDS function. The ggplot2 package was used to visualize the clustering of bacterial communities according to sample types.

3 Results

Beta-diversity analysis (PERMANOVA; Bray–Curtis distance) revealed different bacterial community structures for the different feeds ($P < 0.001$; Table 3). Visualization in NMDS plots demonstrated greater dissimilarities between the milk replacer and the cereal-based diets (i.e., gestation, lactation, and prestarter diets; Figure 1A) than among the cereal-based diets. By contrast, gene copy numbers of total bacteria and alpha-diversity indices were similar among the four feeds (Table 4). The taxonomic composition supported different core genera for the cereal-based diets and the milk replacer. Dominant genera found in the gestation and lactation diets were *Pantoea*, *Paenibacillus*, *Pseudomonas*, and *Erwinia*, which accounted for 67.9% and 59.3% of all reads for the gestation and lactation diets, respectively (Figure 2A; Supplementary Table 1). *Pantoea*, *Paenibacillus*, and *Pseudomonas* also belonged to the core genera in the prestarter diet, with *Pantoea* being 44% less abundant in the prestarter compared to the gestation diet ($P < 0.05$). Compared to the diets for sows, the prestarter diet contained higher relative abundances of *Lactococcus* and *Sphingomonas*. The milk replacer was mainly dominated by *Streptococcus* and *Lactococcus* (57.4% and 13.2% of all reads, respectively). *Pantoea, Paenibacillus*, and *Pseudomonas* were also among the 10 most abundant genera in the milk replacer but in much lower abundances compared to the cereal-based gestation and lactation diets ($P < 0.001$). Notably, the relative abundance of *Fructilactobacillus* was higher in the lactation diet compared to the other three feeds ($P < 0.05$). At the ASV level for the five most abundant genera in the feeds, the data showed that there were four

TABLE 3 Permutational multivariate analysis of variance (PERMANOVA) results for bacterial communities in the feed.

Source of variation	df	Sum of squares	R^2	F	*P*-value
Bacteria					
Feeds	6	0.86	0.86	14.76	0.001
Residual	15	0.15	0.15		
Total	21	1.00	1.00		
Milk replacer and stomach digesta day 3 to 21	4	3.87	0.40	13.06	0.001
Residual	78	5.77	0.60		
Total	82	9.64	1.00		
Prestarter diet and stomach digesta day 28 to 35	3	3.71	0.45	16.24	0.001
	60	4.57	0.55		
	63	8.27	1.00		

Df, degrees of freedom; F, F-value by permutation. The analysis based on pairwise distance of a multivariate data set and values were obtained using type III sums of squares with 999 permutations of residuals, considering significant difference at $P \leq 0.05$.

Pantoea ASV with *P. vagans* and *P. agglomerans* being the main contributing ASV (Figure 3A). *Paenibacillus* was represented by three ASV, of which *P. hordei* was the most abundant in all feeds (Figure 3B). Moreover, feeds contained between nine and eleven *Pseudomonas*-ASV with *P. syringae* and *P. rhizosphaerae* being the most abundant ASV in all four feeds (Figure 3C). As with the whole genus, they were more abundant in the gestation, lactation (as a trend), and prestarter diets compared to the milk replacer ($P < 0.05$). By contrast, the high abundance of the genus *Streptococcus* in the milk replacer was mainly due to the high abundance of *S. salivarius* (59.4% of all reads; Figure 3D), whereas the other three *Streptococcus* ASV each represented only 1% or less of all reads in all feeds. The genus

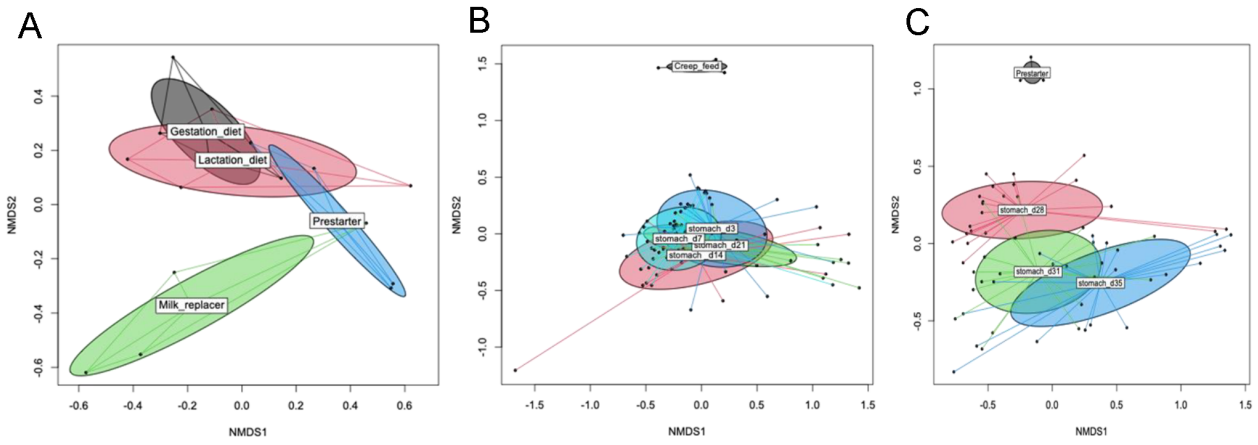

FIGURE 1
Beta-diversity of bacterial communities in feeds for sows and piglets **(A)**; milk replacer and gastric digesta on days 3, 7, 14, and 21 of life **(B)**; and prestarter diet and gastric digesta on days 28, 31, and 35 of life **(C)**. Non-metric multidimensional scaling (NMDS) based on Bray–Curtis distance.

TABLE 4 Comparison of total bacterial gene copy numbers, species richness and diversity among feeds for sows and piglets.

Item	Gestation diet	Lactation diet	Milk replacer	Prestarter diet	SEM	*P*-value
Total bacteria (log_{10} gene copies/g)	9.0	9.0	8.5	8.3	0.27	0.252
Observed features	301	353	328	369	77.01	0.929
Shannon	2.14	2.14	2.03	2.42	0.181	0.494
Simpson	0.71	0.71	0.70	0.75	0.025	0.540

Values are least-squares means ± standard error of the mean (SEM).

Lactococcus was represented by only one ASV (*L. lactis*) in all four feeds and was significantly higher in the milk replacer and prestarter than in the gestation and lactation diets ($P < 0.05$; Figure 3D).

The detailed bacterial composition in the gastric digesta of piglets on days 3, 7, 14, 21, 28, 31, and 35 of life can be found in Yosi et al. (2024). Briefly, dominant genera in the gastric digesta of piglets were *Lactobacillus*, *Limosilactobacillus*, *Lactobacillaceae* HT002, *Streptococcus*, and *Actinobacillus* during the suckling phase, and *Lactobacillus*, *Ligilactobacillus*, *Terrisporobacter*, *Lactobacillaceae* HT002, and *Ralstonia* after weaning (Yosi et al., 2024). Moreover, the intake of the milk replacer and prestarter diet, estimated on a pen basis, is presented in Yosi et al. (2024). The intake of milk replacer and prestarter diet ranged from an average of 16.5 to 23.5 g dry matter/day per piglet from day 3 to 28 of life.

Comparison of the bacterial communities (beta-diversity) in the milk replacer with those in gastric digesta on days 3, 7, 14, and 21 of life (Figure 1B), as well as of the bacterial communities in the prestarter diet with those in gastric digesta on days 28, 31, and 35 of life (Figure 1C), showed distinct clustering between feed and gastric digesta, as illustrated in the NMDS plots and confirmed by PERMANOVA (Table 3). When comparing the relative genera abundances in gastric digesta with those in the milk replacer and prestarter diet, most genera that were highly abundant in feed were also detected in gastric digesta and showed changes in abundance with increasing age of the piglets ($P < 0.05$; Figure 2B).

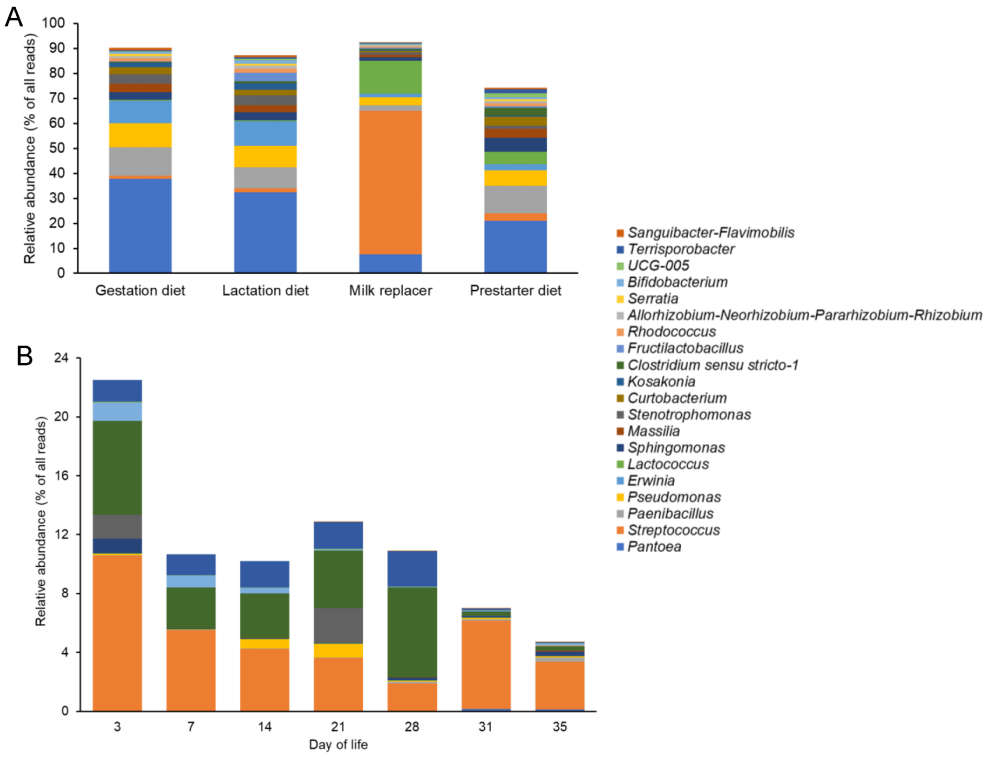

FIGURE 2
Relative abundances (% of all reads) of the 20 most abundant genera in feeds for sows and piglets **(A)** and their abundances in gastric digesta of piglets on days 3, 7, 14, 21, 28, 31, and 35 of life **(B)**. Values are least squares means ± standard error of the mean. The genus at the bottom of the legend corresponds to the genus at the bottom of the stacked bar chart. P values for diet-type effects on genera abundances in feeds are in Supplementary Table S1. P values for age effects on genera abundances in gastric digesta are in Supplementary Table S2. Data for bacterial genera were adapted from Yosi et al. (2024).

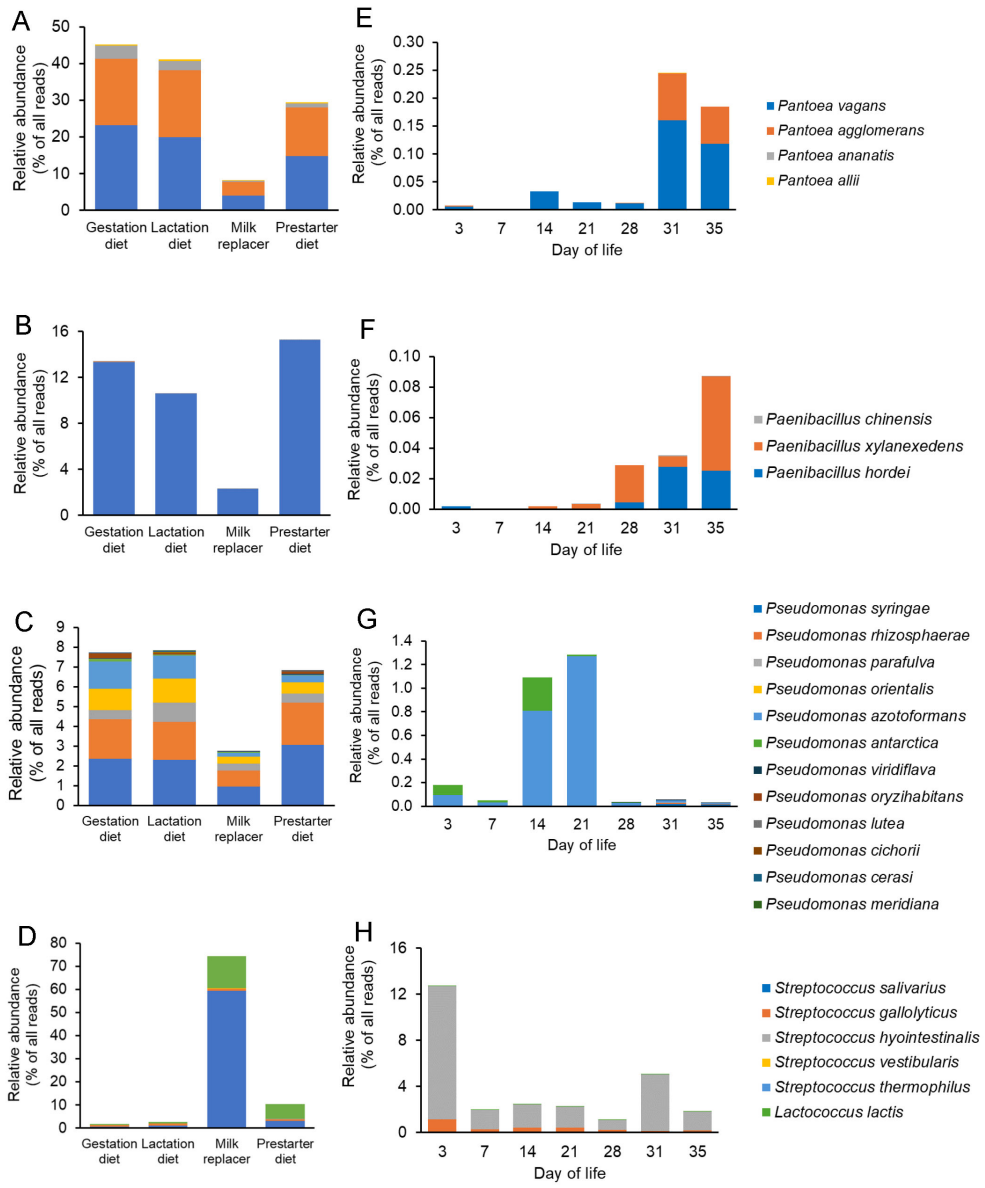

FIGURE 3
Relative abundances (% of all reads) of the amplicon sequence variants (ASV) within the five most abundant genera in feeds for sows and piglets. Pantoea ASV **(A)**, Paenibacillus ASV **(B)**, Pseudomonas ASV **(C)**, and Streptococcus/Lactococcus ASV **(D)** in feeds; and Pantoea ASV **(E)**, Paenibacillus ASV **(F)**, Pseudomonas ASV **(G)**, and Streptococcus/Lactococcus ASV **(H)** in gastric digesta of piglets on days 3, 7, 14, 21, 28, 31, and 35 of life. Values are least squares means ± standard error of the mean. P values for diet-type effects on genera abundances in feeds are in Supplementary Table S1. P values for age effects on genera abundances in gastric digesta are in Supplementary Table S2.

Comparing the relative abundances of ASV representing the five most abundant genera in feed and gastric digesta (Figures 3E–H) also showed age-related development of the relative ASV abundances. Accordingly, relative abundances of *P. vagans* and *P. agglomerans* increased on days 31 and 35 of life compared to the suckling period ($P < 0.05$; Figure 3E), but their proportional share was less than 0.2% of all reads. Similarly, *P. hordei* and *P. xylanexedens* increased on days 28, 31, and 35 of life compared to the earlier days ($P < 0.05$; Figure 3F). However, both ASV were low in abundance, with relative abundances of less than 0.04%, 0.04%, and 0.1% on days 28, 31, and 35 of life, respectively.

By contrast, the relative abundance of *P. azotoformans* was considerably higher on days 14 and 21 of life, contributing more to the *Pseudomonas* community in gastric digesta than on other days ($P < 0.05$; Figure 3G), reaching 0.8% and 1.3% of all reads in gastric digesta. Among the *Streptococcus* ASV detected in the feed (Figure 3H), mainly *S. hyointestinalis* and *S. gallolyticus* were present in gastric digesta and were more abundant on day 3 of life compared to other days ($P < 0.05$), when they accounted for about 12% of all reads. The *Lactococcus* ASV (*L. lactis*) was detected at relative abundances of less than 0.03% of all reads on all days of life.

4 Discussion

As anticipated, the cereal-based feeds and the milk replacer contained a considerable number of gene copies per gram of feed and a diverse bacterial community. Comparison of the bacterial microbiota in piglets' feed and gastric digesta during the suckling and early postweaning period indicated distinct clustering of the bacterial communities between feed and digesta, indicating low influence of the feed-associated taxa on the overall gastric community composition. Nevertheless, taxonomic comparison confirmed that the dominant taxa in the piglet feed were detectable in gastric digesta, especially when solid feed intake increased after weaning. Therefore, the results from this pilot study support that it is worth considering bacterial DNA originating from pig feed as a potential influencing factor in studies on the porcine gut microbiota. Suggestions for future research on this topic include assessing whether feed-associated microbial DNA can be detected along the gastrointestinal tract and the ratio of genetic material originating from dead or live microbes. The latter may help disentangle whether facultative anaerobic bacteria present in the feed are able to colonize the piglet gut or merely pass through transiently.

The present results for bacterial load, species richness, and diversity among the three cereal-based feeds may support dense colonization of the plants, including cereals, legumes, and tubers used in the feeds. Plants comprise numerous epi- and endophytes, which often act as symbionts and increase the resistance of the host plants toward environmental stressors (Xin et al., 2018; Abdullaeva et al., 2021; Crosby et al., 2023). The cereal-based diets (i.e., gestation, lactation, and prestarter diets) were dominated by *Pantoea*, *Paenibacillus*, *Erwinia*, *Pseudomonas*, and *Sphingomonas*, which have been described as dominant taxa in cereal-based diets before (Sabillón and Bianchini, 2016; You et al., 2021). From the ingredients, the gestation and lactation diets were more similar compared to the prestarter diet, explaining their more similar bacterial compositions and community structures. We could not sample the single ingredients at the commercial feed mill that produced the feed and thus cannot trace back the taxa to the ingredients they came with. However, the higher abundance of *Fructilactobacillus* in the lactation diet compared to the gestation diet may be related to the inclusion of 12.8% bakery products in the lactation diet. Accordingly, *Fructilactobacillus* is a genus strongly associated with sourdough fermentation (Gänzle and Zheng, 2019). Other environmental sources for bacterial contamination were likely storage areas for the raw materials at farms, transportation, and processing plants. Accordingly, the presence of the *Allorhizobium–Neorhizobium–Pararhizobium–Rhizobium* group among the dominant genera in the cereal-based feeds may be indicative of a stronger environmental influence (e.g., contamination with bacteria from the soil) on the microbiota composition in these feeds (Thompson et al., 2021; Arellano et al., 2023).

Although the milk replacer also contained plant-based feedstuffs and wheat starch was the first ingredient, its bacterial community separated from those of the cereal-based diets, as illustrated in the NMDS plots. The milk replacer also contained fermented milk products as major ingredients. Hence, the separation of the bacterial communities from the other feeds can likely be linked to the whey powder and whey protein concentrate (Kleerebezem and de Vos, 2011; Thompson et al., 2021; Kuttappan et al., 2025). The prestarter diet also contained whey powder, but in a lower amount than the milk replacer, explaining the closer clustering of their bacterial communities compared to those of the sow feeds. The family *Streptococcaceae* has been reported to be the most abundant family in whey protein concentrate (Thompson et al., 2021), and *Lactococcus* and *Streptococcus* are the most commonly used starter cultures in cheese production (Kleerebezem and de Vos, 2011; Kuttappan et al., 2025). Accordingly, these two genera accounted for more than 70% of all reads detected in the milk replacer. Despite this dominance, species richness and diversity were similar between the milk replacer and cereal-based diets, which can be attributed to the high numbers of low-abundant taxa in the milk replacer, originating from the contained plant feedstuffs. Taxa assignment at the ASV level designated most of the streptococci to *Streptococcus salivarius* (59.4% of all reads) and, to a lesser extent, to *S. gallolyticus* (1.0% of all reads). However, the salivarius group of streptococci consists of three genetically similar species, namely *S. salivarius*, *S. vestibularis*, and *S. thermophilus* (Delorme et al., 2015), of which only *S. thermophilus* is widely applied as a food bacterium in dairy production (Delorme et al., 2015; Solieri et al., 2022), whereas *S. salivarius* and *S. vestibularis* are commensals in the gut (Kaci et al., 2014; Delorme et al., 2015). This raises the question of whether the three species were appropriately assigned at the 16S rRNA gene level. There is evidence that *S. salivarius* and *S. thermophilus* exhibit high levels of 16S rRNA sequence homology (Bentley et al., 1991), which would support that the streptococci found in the milk replacer are most likely primarily *S. thermophilus* and not *S. salivarius*. We detected *S. thermophilus*, but only as a low-abundant taxon. Only one *Lactococcus* ASV dominated (*Lc. lactis*, 14% of all reads) in the milk replacer, which has been shown to attenuate gut inflammation and gut barrier loss and to inhibit pathogen adhesion in *in vitro* Caco-2 models (Kuttappan et al., 2025; Roselli et al., 2025). Therefore, cheese starter cultures, when consumed with the food, may exert probiotic properties in the gut (Roselli et al., 2025). These are beneficial effects for gut health in young pigs, too, especially around weaning. The question arises whether the bacteria or their metabolites (e.g., bacteriocins) are still active in the piglet feed, as the feeds or components are commonly heat-treated.

The porcine gut microbiota develops during the first weeks of life (Arsenault et al., 2024; Yosi et al., 2024). During this time, microbes present in sow milk influence intestinal colonization. As piglets explore their environment, including additionally offered milk replacer, they take up the associated microbes and their DNA. The intake of creep feed is usually low during the suckling phase and may vary among individual piglets (Lerch et al., 2023; Yosi et al., 2024). Accordingly, in the present study, the average amount of ingested milk replacer amounted to 20 g dry matter/day per piglet (Yosi et al., 2024). To estimate the influence of the offered feed on

gut bacterial composition, we focused on the bacterial microbiota in gastric digesta, as it can be assumed that the carryover of bacterial DNA from feed is more visible in this gut compartment. The data for the pre- and postweaning gastric community support that a carryover of bacterial DNA from the feed occurred, as bacterial genera and ASV found to be dominant in the milk replacer and prestarter diet were present in the gastric microbiota and hence may impact data interpretation. The standard error for certain analyzed, feed-associated ASV in gastric digesta indicated greater variation among piglets, which may correspond to their actual ingestion of milk replacer and prestarter diet. When interpreting gut microbiota results, we generally assume that the detected bacteria are metabolically active, which may not be the case with bacteria originating from the feed due to heat and pressure treatment during feed processing. Hence, dead bacterial cells may be regarded as transient and inactive constituents in the gut, but the opposite may be true. Dead bacterial cells can still interact with the host animal via gut mucosal pattern recognition of bacterial cell surface structures and thus may act as inflammatory stimuli (Shukla and Tangney, 2025) and via their metabolites present in the feed.

According to the present data, the impact of plant-associated taxa like *Pantoea* and *Paenibacillus*, with a proportional share ranging from 0.001% to 0.31% of the gastric bacterial community, on the interpretation of results for gut microbiota development in young piglets may be small. However, interpretation may become more difficult for genera that are commonly recognized as belonging to the commensal gut microbiota in pigs, such as *Streptococcus*, *Clostridium* sensu stricto, or *Pseudomonas*, which constitute a higher proportion of the gut bacterial community (Arsenault et al., 2024; Yosi et al., 2024). For these genera, it may be advisable to compare the taxa composition at the ASV level. In doing so, it is important to consider the limitations regarding proper annotation of bacteria on the basis of the 16S rRNA gene and the difficulty of assigning certain taxa at the species level due to the inability to cultivate them (Plessis et al., 2023). Comparison of the *Streptococcus* ASV in the feed and gastric digesta supported that, due to the small amount of milk replacer consumed by the piglets, the streptococci in the milk replacer had only a small influence on the *Streptococcus* community in gastric digesta. Indeed, *S. salivarius* was very low in abundance in gastric digesta, whereas *S. hyointestinalis*, which was low in abundance in the milk replacer, was high in abundance in gastric digesta. Of note, two *Pseudomonas* ASV, *P. azotoformans* and *P. antarctica*, largely increased in their abundances from day 7 to 14 of life (and day 21 for *P. azotoformans*) and then decreased again on day 28 of life. As the measured intake of milk replacer did not change from day 7 to 14 of life, the question arises whether this increase was related to gut maturational changes (Yosi et al., 2024). Another possibility is that piglets consumed feed drippings from the mouth of the sow as a source for these ASV.

Postweaning, the piglets depended on the prestarter diet as their sole feed. The associated higher intake of solid feed was also visible in the gastric abundance of plant-associated genera, such as *Pantoea*, *Paenibacillus*, and *Erwinia*, but they remained low-abundant taxa. By contrast, the relative abundances of *Streptococcus* and *Pseudomonas* as gut commensals in gastric digesta did not seem to reflect the solid feed intake of piglets after weaning. Consequently, bacterial DNA that is clearly assignable to taxa associated with plant feedstuffs (Sabillón and Bianchini, 2016; You et al., 2021) may be predictive for piglets' feed intake in the early postweaning period, which should be assessed more closely in the future.

In conclusion, the present data demonstrate that sow and piglet feeds comprise a diverse and dense bacterial microbiota with clearly distinguishable profiles between mainly plant-based feeds and the milk replacer. Comparing the bacterial data in the piglet feed with that of gastric digesta showed that, due to the low intake, the influence of feed-associated taxa (milk replacer) was small and rather negligible during the suckling phase. The proportion of bacterial DNA belonging to feed-associated taxa increased postweaning, but their influence remained small. Overall, the relative abundances of *Pantoea* ASV and *Paenibacillus* ASV, as plant-associated taxa, and that of *Lactococcus* appeared to be predictive to a certain degree for the actual intake of milk replacer and prestarter diet, which should be corroborated in future studies. Due to the omnipresence of fungi as endophytes on plants, it will be worth investigating the fungal communities in feeds in future studies.

Data availability statement

The datasets generated for this study were deposited into the NCBI Bioproject databank under accession number PRJNA1098483 (feed) and PRJNA PRJNA1103974 (gastric digesta, Yosi et al., 2024).

Ethics statement

The animal study was approved by institutional ethics committee of the University of Veterinary Medicine Vienna and Austrian national authority in accordance with the Law for Animal Experiments in Austria. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

KH: Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. FL: Investigation, Methodology, Writing – review & editing. FY: Investigation, Methodology, Writing – review & editing. JV: Investigation, Methodology, Writing – review & editing. BM-Z: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research and/or publication of this article. This research was

supported by the Austrian Federal Ministry for Digital and Economic Affairs and the National Foundation for Research, Technology and Development. BIOMIN Holding GmbH, which is part of dsm-firmenich, financially supported the Christian Doppler Laboratory for Innovative Gut Health Concepts of Livestock. Open access funding provided by University of Veterinary Medicine Vienna. The funders were not involved in the study design, collection, analysis, interpretation of data, the writing of this article, or the decision to submit it for publication.

Acknowledgments

We thank J. Ehmig, T. Enzinger (Clinical Department for Farm Animals and Food Systems Science), S. Posseth and T. Strini (VetFarm) for their excellent help with laboratory analysis, animal caretaking and assistance during sampling.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2025.1710910/full#supplementary-material

References

Abdullaeva, Y., Ambika Manirajan, B., Honermeier, B., Schnell, S., and Cardinale, M. (2021). Domestication affects the composition, diversity, and co-occurrence of the cereal seed microbiota. *J. Advanced Res.* 31, 75–86. doi: 10.1016/j.jare.2020.12.008

Arellano, H., Nardello-Rataj, V., Szunerits, S., Boukherroub, R., and Fameau, A-L. (2023). Saturated long chain fatty acids as possible natural alternative antibacterial agents: Opportunities and challenges. *Adv. Colloid Interface* 318, 102952. doi: 10.1016/j.cis.2023.102952

Arsenault, M., Lillie, B., Nadeem, K., Khafipour, E., and Farzan, A. (2024). Progression of swine fecal microbiota during early stages of life and its association with performance: a longitudinal study. *BMC Microbiol.* 24, 182. doi: 10.1186/s12866-024-03336-y

Bentley, R. W., Leigh, J. A., and Collins, M. D. (1991). Intrageneric structure of Streptococcus based on comparative analysis of small-subunit rRNA sequences. *Int. J. Syst. Bacteriol* 41, 487–494. doi: 10.1099/00207713-41-4-487

Bonde, C. S., Drøhse, F. B., Büdeyri Gökgöz, N., Krych, L., Nielsen, D. S., Petersen, H. H., et al. (2025). Dietary supplementation with fermented rapeseed and seaweed modulates parasite infections and gut microbiota in outdoor pigs. *Front. Vet. Sci.* 12. doi: 10.3389/fvets.2025.1565686

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). Dada2: High-resolution sample inference from Illumina amplicon data. *Nat. Meth* 13, 581–583. doi: 10.1038/nmeth.3869

Cheli, F., Battaglia, D., Pinotti, L., and Baldi, A. (2012). State of the art in feedstuff analysis: A technique-oriented perspective. *J. Agric. Food Chem.* 60, 9529–9542. doi: 10.1021/jf302555b

Crosby, K. C., Rojas, M., Sharma, P., Johnson, M. A., Mazloom, R., Kvitko, B. H., et al. (2023). Genomic delineation and description of species and within-species lineages in the genus Pantoea. *Front. Microbiol.* 14. doi: 10.3389/fmicb.2023.1254999

Delorme, C., Abraham, A.-L., Renault, P., and Guédon, E. (2015). Genomics of Streptococcus salivarius, a major human commensal. *Infect. Genet. Evol.* 33, 381–392. doi: 10.1016/j.meegid.2014.10.001

Gänzle, M. G., and Zheng, J. (2019). Lifestyles of sourdough lactobacilli - Do they matter for microbial ecology and bread quality? *Int. J. Food Microbiol.* 302, 15–23. doi: 10.1016/j.ijfoodmicro.2018.08.019

Gardiner, G.E., Metzler-Zebeli, B.U., and Lawlor, P.G.. (2020). Impact of intestinal microbiota on growth and feed efficiency in pigs: A review. *Microorganisms* 8, 1886. doi: 10.3390/microorganisms8121886

Jackman, J. A., Boyd, R. D., and Elrod, C. C. (2020). Medium-chain fatty acids and monoglycerides as feed additives for pig production: towards gut health improvement and feed pathogen mitigation. *J. Anim. Sci. Biotechnol.* 11, 44. doi: 10.1186/s40104-020-00446-1

Kaci, G., Goudercourt, D., Dennin, V., Pot, B., Doré, J., Ehrlich, S. D., et al. (2014). Anti-inflammatory properties of Streptococcus salivarius, a commensal bacterium of the oral cavity and digestive tract. *Appl. Environ. Microbiol.* 80, 928–934. doi: 10.1128/AEM.03133-13

Karlsen, C., Tzimorotas, D., Robertsen, E. M., Kirste, K. H., Bogevik, A. S., and Rud, I. (2022). Feed microbiome: confounding factor affecting fish gut microbiome studies. *ISME Commun.* 2, 14. doi: 10.1038/s43705-022-00096-6

Kleerebezem, M., and de Vos, W. M. (2011). Lactic acid bacteria: life after genomics. *Microb. Biotechnol.* 4, 318–322. doi: 10.1111/j.1751-7915.2011.00262.x

Kuttappan, D., Muttathukonam, S. H., and Amalaradjou, M. A. (2025). Cheese starter cultures attenuate inflammation in the *in vitro* Caco-2 model. *AIMS Microbiol.* 11, 369–387. doi: 10.3934/microbiol.2025017

Lerch, F., Yosi, F., Vötterl, J. C., Koger, S., Ehmig, J., Sharma, S., et al. (2023). An insight into the temporal dynamics in the gut microbiome, metabolite signaling, immune response, and barrier function in suckling and weaned piglets under production conditions. *Front. Vet. Sci.* 10. doi: 10.3389/fvets.2023.1184277

Lin, H., and Peddada, S. D. (2020). Analysis of microbial compositions: a review of normalization and differential abundance analysis. *NPJ Biofilms Microbiomes* 6, 60. doi: 10.1038/s41522-020-00160-w

Maciorowski, K. G., Herrera, P., Jones, F. T., Pillai, S. D., and Ricke, S. (2007). Effects on poultry and livestock of feed contamination with bacteria and fungi. *Anim. Feed Sci. Technol.* 133, 109–136. doi: 10.1016/j.anifeedsci.2006.08.006

Metzler-Zebeli, B. U., Lerch, F., Yosi, F., Vötterl, J., Ehmig, J., Koger, S., et al. (2023). Temporal microbial dynamics in feces discriminate by nutrition, fecal color, consistency and sample type in suckling and newly weaned piglets. *Animals* 13, 2251. doi: 10.3390/ani13142251

Muyzer, G., de Waal, E. C., and Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* 59, 695–700. doi: 10.1128/aem.59.3.695-700.1993

National Research Council (NRC) (2012). *Nutrient Requirements of Swine. 10th ed* (National, Washington, DC: Academy Press).

Naumann, C., and Bassler, R. (2012). *Methodenbuch, Band III: Die chemische Untersuchung von Futtermitteln. 3rd ed* (Speyer, Germany: VDLUFA-Verlag).

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2022). *Vegan: Community Ecology Package* (R Package). Available online at: https://cran.r-project.org/package=vegan.

Plessis, C., Jeanne, T., Dionne, A., Vivancos, J., Droit, A., and Hogue, R. (2023). ASVmaker: A new tool to improve taxonomic identifications for amplicon sequencing data. *Plants (Basel)* 12, 3678. doi: 10.3390/plants12213678

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596. doi: 10.1093/nar/gks1219

Roselli, M., ColaFranceschi, F., Cipriani, V., Valle, A., Zinno, P., Guantario, B., et al. (2025). Isolation and characterization of lactic acid bacteria from an italian traditional raw milk cheese: probiotic properties and technological performance of selected strains. *Microorganisms* 13, 1368. doi: 10.3390/microorganisms13061368

Sabillón, L., and Bianchini, A. (2016). From field to table: A review on the microbiological quality and safety of wheat-Based products. *Cereal Chem.* 93, 105–115. doi: 10.1094/CCHEM-06-15-0126-RW

Shukla, A., and Tangney, M. (2025). Bacterial exopolysaccharides and live biotherapeutics: Orchestrating immune modulation and therapeutic potential in the gut microbiome era. *BioMed. Pharmacother.* 191, 118510. doi: 10.1016/j.biopha.2025.118510

Solieri, L., Valentini, M., Cattivelli, A., Sola, L., Helal, A., Martini, S., et al. (2022). Fermentation of whey protein concentrate by Streptococcus thermophilus strains releases peptides with biological activities. *Process Biochem.* 121, 590–600. doi: 10.1016/j.procbio.2022.08.003

Stanley, J. S., Mansbridge, S. C., Bedford, M. R., Connerton, I. F., and Mellits, K. H. (2025). Prebiotic xylo-oligosaccharides modulate the gut microbiome to improve innate immunity and gut barrier function and enhance performance in piglets experiencing post-weaning diarrhoea. *Microorganisms* 13, 1760. doi: 10.3390/microorganisms13081760

Tardiolo, G., Daghio, M., Riggio, V., Zumbo, A., Virga, N. A., and Sutera, A. M. (2025). Faecal microbiota differences between an autochthonous pig breed and a commercial line. *Sci. Rep.* 15, 32176. doi: 10.1038/s41598-025-13460-y

Thompson, C. M., Gao, Q., Chang, P., and Swanson, G. (2021). Differentiation of milk and whey protein concentrates by microbiome profiling using 16S metagenomics. *J. AOAC Int.* 104, 757–764. doi: 10.1093/jaoacint/qsaa158

Xin, X.-F., Kvitko, B., and He, S. Y. (2018). Pseudomonas syringae: What it takes to be a pathogen. *Nat. Rev. Microbiol.* 16, 316–328. doi: 10.1038/nrmicro.2018.17

Yosi, F., Lerch, F., Vötterl, J. C., Koger, S., Verhovsek, D., and Metzler-Zebeli, B. U. (2024). Lactation-related dynamics of bacterial and fungal microbiomes in feces of sows and gut colonization in suckling and newly weaned piglets. *J. Anim. Sci.* 102, skae321. doi: 10.1093/jas/skae321

You, Y., Aho, K., Lohse, K. A., Schwabedissen, S. G., Ledbetter, R. N., and Magnuson, T. S. (2021). Biological soil crust bacterial communities vary along climatic and shrub cover gradients within a sagebrush steppe ecosystem. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.569791